

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification 6 : A61K 38/00, 39/00, 39/385, 31/44, 31/415, 31/425</p>	<p>A1</p>	<p>(11) International Publication Number: WO 99/59612 (43) International Publication Date: 25 November 1999 (25.11.99)</p>
<p>(21) International Application Number: PCT/US99/10734 (22) International Filing Date: 14 May 1999 (14.05.99) (30) Priority Data: 60/085,610 15 May 1998 (15.05.98) US (71) Applicant (for all designated States except US): APHTON CORPORATION [US/US]; Brickell Bay View Center, Suite 2160, 80 S.W. Eighth Street, Miami, FL 33130 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): GEVAS, Philip, C. [US/US]; 881 Ocean Drive #23D, Key Biscayne, FL 33149 (US); GRIMES, Stephen [US/US]; 551 Rutgers Drive, Davis, CA 95616 (US); KARR, Stephen [US/US]; 2265 Halsey Circle, Davis, CA 95616 (US); MICHAELI, Dov [US/US]; 21 Marina Vista Avenue, Larkspur, CA 94939 (US). (74) Agents: BAK, Mary, E. et al.; Howson and Howson, Spring House Corporate Center, P.O. Box 457, Spring House, PA 19477 (US).</p>		<p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report.</p>
<p>(54) Title: METHOD FOR THE TREATMENT OF GASTROESOPHAGEAL REFLUX DISEASE (57) Abstract A method for the treatment of gastroesophageal reflux disease comprising a combination of active immunization with an anti-gastrin immunogenic composition with an antagonist which blocks or inhibits the gastric acid pump activity; or alternatively administering purified anti-gastrin antibodies with a H₂ antagonist or proton pump inhibitor of the gastric acid producing enzyme system.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

METHOD FOR THE TREATMENT OF GASTROESOPHAGEAL REFLUX DISEASE

The present invention relates to a combination of immunological, antihormonal and enzyme inhibitory methods for the treatment of gastroesophageal reflux disease.

BACKGROUND OF INVENTION

Gastroesophageal reflux disease ("GERD") is a common and chronic disorder which requires long-term, even lifelong, therapy. GERD is commonly known as heartburn, and is characterized by a retrosternal burning sensation, and regurgitation of the stomach contents. About 40% of adults in the United States have experienced occurrences of the disease, and approximately 10% have daily troubling symptoms.

GERD occurs when there is an abnormally prolonged contact time between the esophageal mucosa and refluxate, which is believed to be primarily gastric acid (DeVault, et al., *Mayo Clinic Proc.* 69:867-876, 1994 and Redmond, et al. In "Gastroesophageal Reflux Disease" Ronald Hinder ed., R.G. Landes Co., Ch. 1, pages 1-6, 1993). The regurgitation of the gastric contents and duodenal juice is believed to be due to either an incompetent lower esophageal sphincter or more frequently to an inappropriate sphincter relaxation at the time of transfer of the stomach contents between stomach and small intestine. The resultant reflux of acid and other materials from the stomach may induce pain or damage the esophageal mucosa. This damage to the esophageal mucosa may lead to esophagitis which is characterized by inflammation of the esophageal mucosa, bleeding, cytological changes, peptic esophageal stricture, esophageal ulcer and Barrett's metaplasia, depending on the severity of the disease.

Gastric acid is produced by parietal cells in the stomach upon stimulation by acetylcholine, histamine and gastrin following the binding of each of these compounds with specific receptors on the surface of the cells. The peptide hormone gastrin is produced by mucosal cells in the stomach. Gastrin is secreted into the blood stream, and is the most potent stimulant of acid secretion by the parietal cell. Gastrin is present in two molecular forms, heptadecagastrin (G17) and tetratriacontagastrin (G34). G17 is the primary stimulator of meal-induced gastric acid

secretion and is 1500 times more potent than histamine, accounting for 60% of the gastrin-mediated acid release. It has also been found that in GERD patients having an abnormal sphincter, the postprandial levels of gastrin are twice those of a normal person and remain high, beyond 3 hours after the meal (Wetscher, et al. In *Gastroesophageal Reflux Disease*, R.A. Heinder, ed. R.G. Landes Co., Ch. 2, pages 7-29, 1993).

Normal esophageal pH is greater than pH 4. The acid refluxate from the stomach lowers the pH value of the esophagus to less than 4, which results in damage to the esophageal mucosa and the development of GERD. In normal individuals, acidic refluxate is cleared by elimination of the refluxate by peristalsis of the esophagus and by neutralization of the acid with the bicarbonate produced by submucosal esophageal glands, and the bicarbonate present in swallowed saliva. In GERD patients, these mechanisms of acid neutralization are not sufficient to restore the normal esophageal pH values and prevent mucosal damage, since reflux of stomach contents occurs more frequently, and for a more prolonged period of time, than in normal individuals (Booth, et al., *Arch. Surg.* 96: 731-734, 1968 and Demeester, et al., *Ann. Surg.* 184: 459-470, 1976). Since it is not medically practical to alter the esophageal acid neutralization mechanisms, GERD therapies are directed to raising the pH of the stomach contents.

Currently, various therapies are available for the treatment of GERD. Historically, the medical treatment for GERD consisted of using antacids as acid neutralizing agents or anti-refluxants, such as alginates, for alleviating an acute onset of the disease. However, these treatments are not effective for the therapy of chronic and severe symptoms of GERD. Systemic medications currently used for treating GERD include the histamine receptor antagonists, cimetidine and ranitidine, which are acid-suppressive agents directed to the inhibition of the four histamine type 2 ("H₂") receptors. These agents prevent the normal binding of histamine, thereby inhibiting the parietal cell from secreting gastric acid and thus, they increase the pH of the stomach contents. The most commonly used histamine H₂ - antagonists are cimetidine hydrochloride (Tagamet®, SmithKline Beecham Pharmaceuticals), ranitidine hydrochloride (Zantac®, Glaxo Pharmaceuticals), famotidine (Pepcid®, Merck & Co.) and nizatidine (Eli Lilly & Co.). The use of these H₂ antagonists is the standard

treatment of acid-caused peptic disorders including GERD, since surgery, a more radical approach, is usually contraindicated.

Despite the widespread acceptance of histamine H_2 receptor blockers, controlled studies on GERD patients treated with these acid inhibiting compounds have yielded variable results on the healing of esophagitis and persistent symptomatic responses, such as continued acid production in the stomach. Studies using cimetidine and ranitidine in GERD patients, at doses and durations that had been proven effective in healing peptic ulcers, were not effective in GERD (Sabesin et al. *Arch. Intern. Med.* 151:2394, 1991). At higher doses and duration of the H_2 -antagonist therapy (400-800mg and 150-300mg twice daily, respectively, for cimetidine and ranitidine), approximately 50-70% (mean, 61%) of patients had symptomatic relief of GERD, and 0 to 82% (mean, 48%) had healing of their esophagitis as endoscopically determined (DeVault, et al. *ibid*; Koelz, H.R. *Scand. J. Gastroenterol.* 24:25-26, 1989 and Fennerty and Sampliner, *Arch. Intern. Med.* 151:2365-2366, 1991).

The healing of ulcerated or eroded esophageal mucosa requires a longer and more profound acid suppression than is necessary in treating other gastrointestinal ulcers. In patients in whom the symptoms of GERD disappeared after an effective treatment with histamine blockers, the symptoms of the disease reappeared soon after the treatment was discontinued (Antonson, et al. *Gastroenterology* 98: A16, 1990 and Bardhan, et al. *Gastroenterology* 98: A18, 1990).

Many patients with severe GERD hypersecrete gastric acid and may require high doses of H_2 antagonists, which become problematic in terms of patient compliance and long term use of these agents. The high doses of H_2 blockers when given to patients for a long period of time may cause undesirable side effects such as, blood pressure and heart problems. The increase in the effective dosage required to bring about relief of GERD symptoms results in very costly therapy. Although treatments of esophagitis vary widely depending on the severity of the disease, the more severe, high-grade types of the disease respond poorly to standard doses of histamine blockers. Approximately 50% or more of patients with GERD do not respond to histamine H_2 antagonist therapy and still require some other form of treatment. In addition, the effective treatment of GERD not only depends on increasing the concentration of histamine blockers or the hydrogen pump inhibitors

which have also been found to be effective in the treatment of GERD, but effective dosaging must be frequent, since the compounds have limited transient time in the patient and must be given in some situations approximately 4 times daily. In a significant number of cases, the patient is not responsive to H₂ blockers.

5 Proton pump inhibitors omeprazole (Astra AB), or anti-H⁺/K⁺-ATPase enzyme inhibitory compound, as well as its analogue, lansoprazole, (Takeda Chemicals) or pantoprazole (Byk Gulden) which inhibit acid secretion in the stomach by inhibiting the proton (hydronium ion) pump mechanism for producing hydrochloric acid in the parietal cell, have been found to be more effective than histamine H₂ blockers in alleviating the symptoms of GERD esophagitis. The resulting increase in pH induced by omeprazole leads to approximately 62-94% (mean, 83%) in symptomatic relief and a healing of the esophagitis occurs in 71-96% (mean, 78%) of the patients in 4-8 weeks of treatment for GERD, almost twice that of ranitidine (DeVault et al. *ibid*; Zeitoun, P. *Scand. J. Gastroenterology* 166 (Suppl.): 83, 1989).

10 A disadvantage of using omeprazole, lansoprazole, or pantoprazole similar to the case with histamine blockers, is that the compound must be administered at higher doses (20 mg, twice daily or 40 mg once daily) than the dosages required to treat gastric and duodenal ulcers (20mg, once daily), and for a longer period of time in order to effectively treat GERD.

20 Furthermore, the prolonged use of histamine blockers or omeprazole for the treatment of GERD results in an increase in serum gastrin levels (2 to 4 times the basal rate). It has been suggested that the increase in gastrin levels could lead to undesirable side effects such as dangerous trophic effects on the human gastric mucosa (Festen, et al. *Gastroenterology* 87: 1030-1034, 1984; Jansen, et al. *Gastroenterology* 99: 621-628, 1990 and Sontag, et al. *Gastroenterology* 102-109, 1992).

25 Co-assigned U.S. Patents No. 5,023,077 and No. 5,609, 870 disclose immunogenic compositions useful for controlling gastrin levels in a patient by generating anti-gastrin antibodies. Thus U.S. Patent No. 5,023,077 and No. 5,609,870 disclose that the immunogenic compositions are useful for the treatment of gastric and duodenal ulcers and gastrin induced or responsive cancers and the disclosures are hereby incorporated entirely by reference into the present description.

30

There remains a need in the art for additional methods and compositions for the successful therapy of GERD.

SUMMARY OF THE INVENTION

The invention combines a method for reducing gastric acid in the stomach by inhibiting the enzyme responsible for gastric acid production or secretion of gastric acid and an immunological method for reducing or preventing the increase of circulating gastrin. It is the object of the present invention to use anti-gastrin immunogenic compositions in the therapy of GERD in combination with administering effective doses of a proton pump inhibitor or H₂ antagonist so as to substantially raise the gastric pH while preventing elevated levels of circulating blood gastrin hormone.

This invention is directed to the treatment of GERD by gastric acid suppression by administration of a proton pump inhibitor or H₂ blocker together with the immunological reduction of circulating gastric hormone by neutralization of heptadecagastrin G17 or tetratriacontagastrin G34, or both G17 and G34 either by administration of exogenous specific antibodies or *in situ* by an immunogenic composition against gastrin.

It is the preferred embodiment of the invention to treat a patient with GERD by administering effective omeprazole dosages with effective dosages of antagastrin G17 antibodies.

It is the more preferred embodiment to keep the frequency of anti-gastrin immunogen parenteral administration to a patient suffering from GERD at a single effective dose or at least at only a few doses thereof.

Yet another preferred method of the invention is to pre-treat the GERD patient with gastrin immunogen or anti-G17 antibodies before administering the gastric acid producing enzyme inhibitor (i.e. proton pump inhibiting compound).

In one embodiment, the invention concerns a combination therapy with a histamine H₂ antagonist, such as ranitidine, cimetidine, famotidine or nizatidine, or a proton pump inhibitor such as, omeprazole or lansoprazole, using standard dosing procedures for H₂ antagonist or proton pump inhibitor, respectively, as described by the art. In the preferred combination therapy, a patient is actively immunized with an immunological composition comprising gastrin 17(1-9)-h(G17)ser9-Diphtheria Toxin

(see U.S. Patent Nos. 5,023,077 and 5,468,494 (co-assigned)). Once the patient is immunized, histamine H₂ antagonist or proton pump inhibitor therapy is administered for 2-12 weeks or until the desired serum anti-gastrin 17 antibody titer is reached. The novel combination therapy provides a more effective method for controlling acid output by the stomach, since acid production is thus controlled by two independent mechanisms, which results in a more effective method for treating GERD, including the more severe cases of the disease. In addition, the therapy would be a less costly method for treating GERD, without the problems with patient compliance associated with long term standard therapies. Furthermore, the high gastrin levels associated with standard therapies, particularly with omeprazole, are neutralized, and thus, the undesirable side effects are reduced.

The method of this invention for treating GERD permits a reduced dosage of the acid reducing agent both at the acid producing level as well as the acid production stimulating level (gastrin). This reduction of dosages is desirable in the usually prolonged treatment.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 illustrates experimental data concerning the percentage of time that the gastric contents remain above pH 3 in different groups of pigs treated with ranitidine (4 animals), omeprazole (5 animals) and hG17(1-9) (4 animals), as compared to six (6) control animals.

FIGURE 2 illustrates the percentage of time that the gastric contents remain above pH 4 in a group of untreated (control) pigs (5) and groups of pigs treated with human gastrin 17(1- 9)Ser9-Diphtheria Toxin (4 animals), ranitidine (4 animals) and omeprazole (5 animals) as described in **Figure 1**.

FIGURE 3 depicts the baseline median pH of the gastric contents of a group of six (6) untreated (control) pigs and groups of four (4) pigs treated with human G17(1-9)Ser9-Diphtheria Toxoid, three (3) pigs with ranitidine and five (5) pigs with omeprazole as described in **Figure 1**.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a novel combination of methods for the treatment of gastroesophageal reflux disease. The combined method on the one hand comprises inhibiting the normal binding of the hormone gastrin 17 to its physiological receptor by actively immunizing the patient against his or her own gastrin 17 hormone. Alternatively or additionally, the hormone gastrin 34 can be neutralized by active or passive immunization with G34 or C-terminal G17 peptide fragment. On the other hand, the method provides inhibition of production of gastric acid either by proton pump inhibition or H_2 receptor blockage.

The invention provides a novel immunological method for the treatment of gastroesophageal reflux disease using a peptide immunogen which raises sufficient gastrin 17 or gastrin 34 antibody levels in a patient so as to affect the binding of the gastrin 17 or gastrin 34 to its physiological receptors in the patient and raise the pH of the stomach. Gastric acid secretion in the stomach can thus be controlled. The pH of the stomach contents is simultaneously raised to a sufficient pH level, e.g., greater than pH 3 for a prolonged and sufficient period of time to alleviate the GERD symptoms and heal the acid-induced esophagitis. According to the invention, anti-G17 antibodies are induced in the patient by active immunization with peptide immunogens which comprise a G17 immunogen conjugated to an immunogenic carrier. The antibodies raised in the patient by the immunogens selectively and specifically bind gastrin hormone G17 or G34 or both, and neutralize and inhibit separately or together the normal binding of gastrin G17 or G34 or both to its receptors in the parietal cells, thereby controlling acid output in the stomach and preventing gastric acid damage of the esophageal mucosa during regurgitation.

A preferred embodiment of the inventive method provides a single administration of an active gastrin 17 or G34 immunogen, which has several advantages over the standard therapies of the art for treating GERD in that problems with patient compliance and undesirable side effects as a result of the therapy are eliminated. Other advantages of using the immunological methods for the treatment of GERD include the use of a limited number of administrations. A single primary administration with appropriately spaced boosters may last for approximately 6 months to a year. Another advantage is that, in a combination therapy with H_2 agonists or

proton pump inhibitors, effective anti-gastrin 17 antibody titers can be maintained by occasional booster shots while the gastric acid inhibitor dosing is reduced or discontinued. Another advantage of this invention is that the maintenance of antigastrin antibody titers reduces or prevents excessive levels of gastrin in hypogastrinemia which would otherwise result from administration of a proton pump inhibitor or H₂ blocker. A booster shot of the immunological composition prolongs anti-gastrin 17 immunity and gastric acid suppression. Still another advantage of this method is that the immunization allows a sufficient time for the esophagitis to completely heal. Additionally, no surgery is required. Yet another advantage is that combination therapy is more useful for treating severe cases of GERD, without causing undesirable side effects, since excess serum gastrin 17 peptides are physiologically neutralized. In patients where the GERD condition is alleviated, discontinuation of the booster dose may result in resumption of normal gastrin levels.

According to the method of the invention, an immunogen is prepared using peptides or chemical structures that mimic the amino terminal end of gastrin 17 or of gastrin 34. The immunogens and immunogenic compositions of the invention are those described in U.S. Patent No. 5,023,077, U.S. Patent No. 5,469,494 and U.S. Patent No. 5,609,870. The disclosures of these issued patents are hereby incorporated by reference in their entirety. U.S. Patent Nos. 5,023,077, 5,469,494, and 5,609,870 disclose compositions containing anti-gastrin 17 immunogens as well as anti-gastrin 34 immunogens and methods of using these compositions for the treatment of gastric and duodenal ulcers and gastrin responsive cancers.

In the present invention, effective dosages ranging from 0.1mg to 5g of the immunogenic composition are administered for the treatment of GERD combined with 10-80mg daily dose of omeprazole. An effective dosage of the immunogenic composition is capable of eliciting an immune response in a patient and inducing antibody titer against human gastrin 17 within 1-3 months after immunization.

Effective treatment of GERD according to this method results in maintenance of the pH of the stomach contents above pH 3 or 4, and for a more prolonged period of time than with H₂ antagonist therapy. Maintenance of the stomach pH above 3 or 4 is essential in the treatment of GERD, since refluxate material having a pH below 2.0 causes esophagitis by protein denaturation and cell

damage, and pH values below 2.5 triggers painful episodes in a patient. When the pH is maintained above 2.5, pain perception is almost nonexistent (Smith, et al *Gastroenterology* 96: 683-689, 1989) and damage to the esophageal wall is minimized.

The immunogens and immunogenic compositions of the invention typically induce specific antibody responses after a single administration. However, it may take several weeks or months for antibody titers to rise to the desired levels effective for the treatment of GERD.

Combination therapy with a histamine H₂ antagonist, such as ranitidine, cimetidine, famotidine and nizatidine, or a proton pump inhibitor, such as omeprazole or lansoprazole, is designed so that a GERD patient is immunized with an immunogenic composition of the invention, and administration of H₂ antagonist is provided on a daily basis, at least once a day for the first 2-12 weeks of treatment or until the desired serum level of anti-gastrin 17 antibodies is obtained.

Desired anti-gastrin 17 serum levels range from 10 to 300 pmole/ml. Once the desired serum levels of anti-gastrin 17 antibody titer are obtained as measured by ELISA or RIA, the non-immunological gastric acid inhibiting drug portion of the combination therapy may be reduced or discontinued.

In the following Examples, the anti G17 immunogenic composition, 150mg ranitidine and 60mg omeprazole were administered to pigs and the resulting changes in the pH of the stomach contents before and after treatment were measured. Specifically, following the stomach pH measurements of the untreated control state of each pig, the stomach pH of the same pigs was measured after the animals were treated with either ranitidine, or composition of human gastrin 17(1-9)-h(G17)ser9-Diphtheria Toxoid (Gastrimmune), or omeprazole administered individually and at different times in each of four animals (pigs).

Example 1

Gastrin neutralization was achieved by using the immunological composition Gastrimmune which is composed of the amino terminal domain of gastrin-17 linked, via an amino acid or peptide spacer to diphtheria toxoid which acts as the immunogenic carries. The antibodies raised by virtue of the design of the immunogen, cross-reacted with both amidated and glycine-extended gastrin-17, two known

proliferative forms of gastrin.

Serum antibody titers rose within 2 weeks of the initial immunization to levels with an antigen binding capacity of $> 10^6$ M. The presence of anti-gastrin antibodies within the serum of Gastrimmune-immunized mice was confirmed by using an ELISA. As expected, no bound gastrin levels were detected in animals immunized with control immunogen.

Example 2

As can be seen in FIG. 1 and FIG. 2, the pH of the stomach contents remained above pH 3 or 4 in anti-gastrin 17 immunized pigs for a longer period of time than in the pigs treated with ranitidine. In omeprazole treated pigs the stomach pH was maintained above pH 3 or 4 for a longer period of time than pigs which were treated with ranitidine and anti-G17 immunized pigs.

In addition, FIG. 3 shows the median pH exhibited by the stomach contents of control pigs when compared to ranitidine, anti-G17 immunization and omeprazole treatment. The data shows that the stomach pH is maintained at higher levels in pigs than those treated with ranitidine or anti-G17 immunization therapy. Anti-G17 immunized pigs had a median pH higher than ranitidine treated pigs.

Treatment of the pigs with ranitidine was less effective in preventing acid output from the stomach. Omeprazole treatment highly inhibited acid output. A single administration of anti-gastrin 17 immunization inhibited stomach acid output at a level of effectiveness between ranitidine and omeprazole, and sufficient to reduce the stomach acid output levels and increase the stomach pH for the effective treatment of GERD.

A treatment which combines the gastric acid secretion with proton pump inhibitors or H_2 histamine blockers with the novel immunization by e.g. Gastrimmune can thus result in maintaining favorably raised pH in the stomach. Furthermore, the treatment with occasional, effective boosters of the antigastrin immunogenic composition can eventually, possibly within a few months, obviate any additional treatment with the anti-acid secretion drugs, such as e.g. omeprazole or ranitidine.

One of the possible advantages of the administration of a proton pump

inhibitor or H₂ blocker after immunization with an antigastrin immunogen, as described, resides in the use of lower amounts of the proton pump inhibitor or H₂ blocker for effective lowering of gastrin acid secretion or raising of stomach pH to about 3-4.

5 **Example 3**

The human patient suffering from GERD is immunized with 200 µg - 400 µg of primary i.v. inoculation of G17 (1-9) Ser : DT immunogen composition. After 2 weeks a booster of 100 - 200 µg of the G17 (1-9) Ser : DT composition is similarly administered. When the anti-G17 titer has reached a level of about 10-300 pmole/ml sufficient to lower the serum gastrin level to near normal with a concomitant
10 lowering of gastric acid secretion, about 10-20 mg oral omeprazole preparation is administered daily to further reduce or stabilize the gastric secretion at a level which essentially eliminates or substantially ameliorates the GERD symptoms.

15 **Example 4**

Immunogens capable of inducing specific immune responses to either G17 or to G34 were prepared by standard solid state synthesis methods. Each peptide was characterized as to amino acid content and purity.

Peptides with the following amino acid sequences were synthesized:

20 Peptide 1 - Human G17(1-6) ("hG17(6)"): pGlu-Gly-Pro-Trp-Leu-Glu-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 2 - Human G17(1-5) ("hG17(5)"): pGlu-Gly-Pro-Trp-Leu-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 3 - Human G17(1-4) ("hG17(4)"): pGlu-Gly-Pro-Trp-Arg-Pro-Pro-Pro-Pro-Cys

25 Peptide 4 - Rat G17(1-6) ("rG17(6)"): pGlu-Arg-Pro-Pro-Leu-Glu-Arg-Pro-Pro-Pro-Pro-Cys

Peptide 5 - Human G34(1-6) ("hG34(6)"): pGlu-Leu-Gly-Pro-Gln-Gly-Arg-Pro-Pro-Pro-Pro-Cys

30 Peptide 6 - Human G34(13-22) ("hG34/G17 combination"): Asp-Pro-Ser-Lys-Lys-Gln-Gly-Pro-Trp-Leu-Pro-Pro-Pro-Cys

Each of these peptides were conjugated to amino groups present on a carrier such as Diphtheria toxoid ("DT") via the terminal peptide cysteine residue utilizing heterobifunctional linking agents containing a succinimidyl ester at one end and maleimide at the other end of the linking agent.

5 To accomplish the linkage between any of Peptides 1-6 above and the carrier, the dry peptide was dissolved in 0.1 M Sodium Phosphate Buffer, pH 8.0, with a thirty molar excess of dithiothreitol ("DTT"). The solution was stirred under a water saturated nitrogen gas atmosphere for four hours. The peptide containing reduced cysteine was separated from the other components by chromatography over a G10
10 Sephadex column equilibrated with 0.2 M Acetic acid. The peptide was lyophilized and stored under vacuum until used. The carrier was activated by treatment with the heterobifunctional linking agent, e.g., Epsilon-maleimidocaproic acid N-hydroxysuccinimide ester, ("EMCS"), in proportions sufficient to achieve activation of approximately 25 free amino groups per 10^5 molecular weight of carrier. In the
15 specific instance of diphtheria toxoid, this amounted to the addition of 6.18 mg of EMCS (purity 75%) to each 20 mg of diphtheria toxoid.

Activation of diphtheria toxoid was accomplished by dissolving each 20 mg aliquot of diphtheria toxoid in 1 ml of 0.2 M Sodium Phosphate Buffer, pH 6.45. Aliquots of 6.18 mg EMCS were dissolved into 0.2 ml of Dimethyl Formamide
20 ("DMF"). Under darkened conditions, the EMCS was added dropwise in 50 microliter ("ul") amounts to the DT with stirring. After 2 hours of incubation in darkness, the mixture was chromatographed on a G50 Sephadex column equilibrated with 0.1 M Sodium Citrate buffer, pH 6.0, containing 0.1 mM EDTA.

Fractions containing the EMCS activated diphtheria toxoid were
25 concentrated over a PM 10 ultrafiltration membrane under conditions of darkness. The protein content of the concentrate was determined by either the Lowry or Bradford methods. The EMCS content of the carrier was determined by incubation of the activated carrier with cysteine-HCl followed by reaction with 10 mM of Elman's Reagent 5,5'-dithio-bis (2-nitrobenzoic acid) 10 mM. The optical density difference
30 between a blank tube containing cysteine-HCl and the sample tube containing cysteine-HCl and carrier was translated into EMCS group content by using the molar extinction coefficient of 13.6×10^3 for 5-thio-2-nitro benzoic acid at 412 nm.

The reduced cysteine content (-SH) of the peptide was also determined utilizing Elman's Reagent. Approximately 1 mg of peptide was dissolved in 1 ml of nitrogen gas saturated water and a 0.1 ml aliquot of this solution was reacted with Elman's Reagent. Utilizing the molar extinction coefficient of 5-thio-2-nitro-benzoic acid (13.6×10^3), the free cysteine -SH was calculated. An amount of peptide containing sufficient free -SH to react with each of the 25 EMCS activated amino groups on the carrier was dissolved in 0.1 M Sodium Citrate Buffer, pH 6.0, containing 0.1 mM EDTA, and added dropwise to the EMCS activated carrier under darkened conditions. After all the peptide solution had been added to the carrier, the mixture was incubated overnight in the dark under a water saturated nitrogen gas atmosphere.

The conjugate of the peptide linked to the carrier via EMCS is separated from other components of the mixture by chromatography over a G50 Sephadex column equilibrated with 0.2 M Ammonium Bicarbonate. The conjugate eluted in the column void volume is lyophilized and stored desiccated at 20°C until used.

The conjugate may be characterized as to peptide content by a number of methods known to those skilled in the art including weight gain, amino acid analysis, etc. Conjugates of these peptides and diphtheria toxoid produced by these methods were determined to have 20-25 moles of peptide per 10^5 MW of carrier and all were considered suitable as immunogens for immunization of test animals.

Example 5

Peptide hG17(1-9)-Ser9 was prepared by standard solid state synthesis methods. That peptide contains an amino terminal immunomimic of hG17 followed by a carboxy terminal spacer. This peptide comprises a 9 amino acid immunomimic of hG17 (pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-) followed by the "Ser" spacer (-Ser-Ser-Pro-Pro-Pro-Pro-Cys) attached to amino acid number 9 of the hG17 immunomimic.

The peptide was conjugated to amino groups present on the Diphtheria Toxoid ("DT") immunogenic carrier via the terminal peptide cysteine residue utilizing heterobifunctional linking agents containing a succinimidyl ester at one end and maleimide at the other end of the linking agent essentially as described in Example 4.

5 The immunogenic constructs of this invention include an aminoterminal (1-9) G17 peptide or an aminoterminal (1-6) G34 peptide conjugated via a peptide spacer to an immunogenic carrier. The preferred G17 sequence is pyro-Glu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu and the preferred G34 sequence is pGlu-Leu-Gly-Pro-Gln-Gly-Arg-Pro-Pro-Pro-Pro-Cys. The preferred spacer in both constructs is a Ser-peptide (Ser-Ser-Pro-Pro-Pro-Pro-Cys). The preferred immunogenic carrier is
10 diphtheria toxoid, tetanus toxoid, keylimpet hemocyanin, and bovine serum albumin (BSA). The gastrin immunogen is defined as a conjugate of the pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu peptide sequence, with an amino acid spacer linked to an immunogenic carrier. The preferred gastrin immunogen is defined as a conjugate of
15 the (1-9) amino terminal (pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu) peptide which is linked by peptide spacer to diphtheria toxoid.

 Numerous modifications and variations of the present invention are
20 included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

WE CLAIM:

1. A method for the treatment of gastroesophageal reflux disease, comprising administering to a patient an effective amount of an immunogenic composition which generates anti-gastrin antibodies in the patient which bind to gastrin; and administering to said patient an effective amount of histamine H₂ antagonist or a proton pump inhibitor.
2. The method of claim 1, wherein the immunogenic composition is a G17(1-9)Ser9-Diphtheria Toxoid and a pharmaceutically acceptable carrier.
3. The method of claim 1 wherein the histamine H₂ antagonist is administered to the patient until the antibody titer is 10-300pmole/ml.
4. The method of claim 1, wherein the histamine H₂ antagonist is ranitidine.
5. The method of claim 1, wherein the histamine H₂ antagonist is cimetidine.
6. The method of claim 1, wherein the histamine H₂ antagonist is famotidine.
7. The method of claim 1, wherein the proton pump inhibitor is administered to the patient until the patient's serum anti-G17 antibody titer is 10³M.
8. The method of claim 1, wherein the proton pump inhibitor is omeprazole.
9. The method of claim 1, wherein the proton pump inhibitor is lansoprazole.

10. The method of claim 1, wherein the proton pump inhibitor is pantoprazole.
11. The method of claim 1 or 7, wherein the anti-gastrin immunity is maintained by a periodic booster of the anti-gastrin immunogenic composition.
12. The method of claim 11, wherein the periodic booster is administered for about a year.
13. Use of an effective amount of an immunogenic composition which generates anti-gastrin antibodies in the patient which bind to gastrin and an effective amount of histamine H_2 antagonist or a proton pump inhibitor in the preparation of a two-component composition for the treatment of gastroesophageal reflux disease, wherein said treatment comprises administering said immunogenic composition and said histamine antagonist or proton pump inhibitor to a patient.
14. Use according to claim 13, wherein the immunogenic composition is a G17(1-9)Ser9-Diphtheria Toxoid and a pharmaceutically acceptable carrier.
15. Use according to claim 13 wherein the histamine H_2 antagonist is administered to the patient until the antibody titer is 10-300 pmole/ml.
16. Use according to claim 13, wherein the histamine H_2 antagonist is ranitidine.
17. Use according to claim 13, wherein the histamine H_2 antagonist is cimetidine.
18. Use according to claim 13, wherein the histamine H_2 antagonist is famotidine.

19. Use according to claim 13, wherein the proton pump inhibitor is administered to the patient for until the patient's serum anti-G17 antibody titer is 10^{-4} M.
20. Use according to claim 13, wherein the proton pump inhibitor is omeprazole.
21. Use according to claim 13, wherein the proton pump inhibitor is lansoprazole.
22. Use according to claim 13, wherein the proton pump inhibitor is pantoprazole.
23. Use according to claim 13 or 19, wherein the anti-gastrin immunity is maintained by a periodic booster of the anti-gastrin immunogenic composition.
24. Use according to claim 23, wherein the periodic booster is administered for about a year.
25. A method for the treatment of gastroesophageal reflux disease, comprising administering to a patient an effective amount of purified anti-gastrin antibodies which bind to gastrin; and administering to said patient an effective amount of a histamine H_2 antagonist or a proton pump inhibitor.
26. Use of an effective amount of purified anti-gastrin antibodies which bind to gastrin; and an effective amount of a histamine H_2 antagonist or a proton pump inhibitor in the preparation of a medicament for the treatment of gastroesophageal reflux disease.

1 / 3

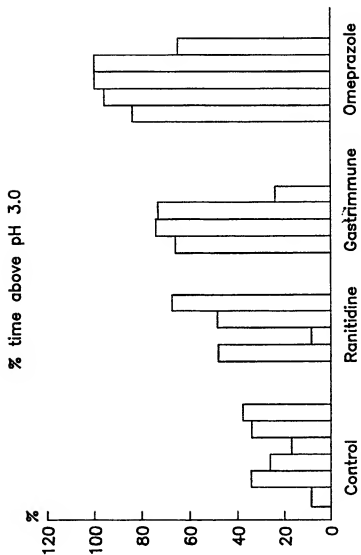


FIG. 1

2/3

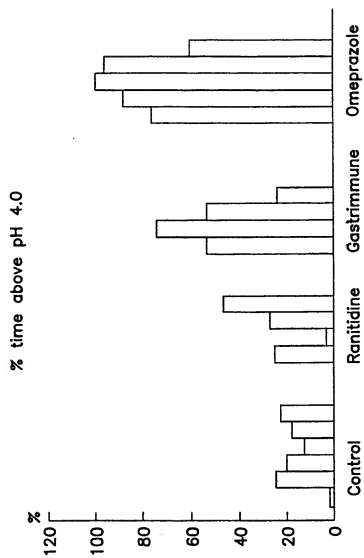


FIG. 2

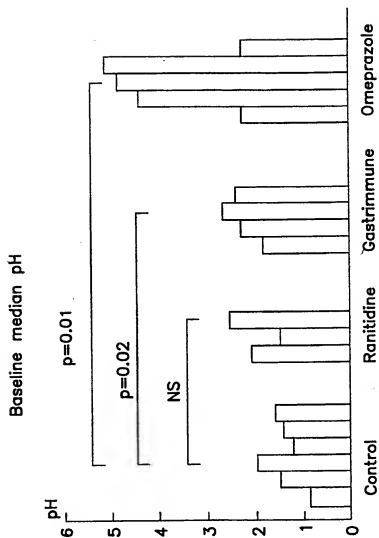


FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10734

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 38/00, 39/00, 39/385, 31/44, 31/415, 31/425

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/13, 14, 15, 16, 17, 18, 338, 339, 370, 400; 424/185.1, 195.11, 197.11, 198.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,468,494 A (GEVAS et al.) 21 November 1995, entire document.	1-26
Y	US 5,609,870 A (GEVAS et al.) 11 March 1997, entire document.	1-26
Y	US 5,023,077 A (GEVAS et al.) 11 June 1991, entire document.	1-26
Y	BUDAVARI, S. The Merck Index (11th Ed.), Rahway, N.J.: Merck & Co., 1989, page 1082.	8-10 and 20-22

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

B earlier document published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

A

document member of the same patent family

Date of the actual completion of the international search

02 JULY 1999

Date of mailing of the international search report

24 AUG 1999

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks

Box PCT

Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized Officer

M. MOEZE

Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10734

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

514/13, 14, 15, 16, 17, 18, 338, 339, 370, 400; 424/185.1, 195.11, 197.11, 198.1